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Opposing patterns of intraspecific and interspecific differentiation in sex chromosomes and autosomes

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Abstract

Linking intraspecific and interspecific divergence is an important challenge in speciation research. X chromosomes are expected to evolve faster than autosomes and disproportionately contribute to reproductive barriers, and comparing genetic variation on X and autosomal markers within and between species can elucidate evolutionary processes that shape genome variation. We performed RADseq on a 16-population transect of two closely-related Australian cricket species, *Teleogryllus commodus* and *T. oceanicus,* covering allopatry and sympatry. This classic study system for sexual selection provides a rare exception to Haldane's rule, as hybrid females are sterile. We found no evidence of recent introgression, despite the fact that the species co-exist in overlapping habitats in the wild and interbreed in the laboratory. Putative X-linked loci showed greater differentiation between species compared to autosomal loci. However, population differentiation within species was unexpectedly lower on X-linked markers than autosomal markers, and relative X-to-autosomal genetic diversity was inflated above neutral expectations. Populations of both species showed genomic signatures of recent population expansions, but these were not strong enough to account for the inflated

X/A diversity. Instead, most of the excess polymorphism on the X could better be explained by sex-biased processes that increase the relative effective population size of the X, such as interspecific variation in the strength of sexual selection among males. Taken together, the opposing patterns of diversity and differentiation at X versus autosomal loci implicate a greater role for sex-linked genes in maintaining species boundaries in this system.

KEYWORDS

hybridisation, faster X effect, population genomics, RAD sequencing, sex chromosomes, *Teleogryllus*

1 | INTRODUCTION

The geographic distribution of genetic variation, within and between species, provides a rich source of information on a species' evolutionary history and the nature of species boundaries (Hewitt, 2001; Sousa & Hey, 2013; Lexer et al., 2013; Gompert et al., 2014). The strength of evolutionary forces can vary across the genome, and specific regions, such as those differing in patterns of inheritance or recombination (e.g. sex chromosomes, mitochondria), might disproportionately contribute to population genetic differentiation and genetic divergence at different scales, for example among species or among populations within species (Charlesworth et al., 1987; Coyne & Orr, 2004; Qvarnström & Bailey, 2009, Nachman & Payseur, 2012). The joint analysis of genetic markers that differ in their pattern of inheritance provides opportunities to identify whether corresponding genomic regions differ in the strength of divergence, and test for evolutionary

processes that might be involved (Shaw, 2002; Payseur et al., 2004; Ségurel et al., 2008; Emery et al., 2010; Lavretsky et al., 2015). Comparisons between autosomes and sex chromosomes are of particular interest. The latter have been suggested to play a key role in the establishment of reproductive barriers and, by extension, speciation (Charlesworth et al., 1987; Sætre et al., 2003; Coyne & Orr, 2004; Presgraves, 2008; Qvarnström & Bailey, 2009), and they have even been described as being "at different stages of speciation" to autosomes in some species comparisons (Ellegren et al., 2012).

Sex chromosomes are expected to evolve faster than autosomes due to asymmetry in their inheritance and differences in patterns of gene expression between the sexes. As a result, selection and drift are predicted to exert different effects on sex chromosomes (Charlesworth et al., 1987; Betancourt et al., 2004; Bachtrog, 2006; Vicoso & Charlesworth, 2006; Ellegren, 2009). Selection is expected to be stronger on X (or Z in female heterogametic species) chromosomes, because recessive mutations are immediately exposed to selection in the heterogametic sex. This is known as the faster-X effect (Haldane 1924, Charlesworth et al., 1987; Meisel & Connallon, 2013). In addition, X chromosomes have an effective population size (Ne) that is only three-quarters that of autosomes (assuming an equal proportion of males and females), so genetic drift is expected to be more pronounced on the X and lead to a higher turnover of alleles (Kauer et al., 2002; Mank et al., 2007; Vicoso & Charlesworth, 2006). The hemizygosity of X chromosomes reduces effective recombination rates, which increases the potential for hitchhiking and selective sweeps (Maynard Smith & Haigh, 1974; Betancourt et al., 2004; Nolte et al., 2013; Garrigan et al., 2014; Dutheil et al., 2015). The latter may be particularly powerful on the X due to higher substitution rates and faster times to fixation (Betancourt et al.,

2004). Increased linkage disequilibrium on the sex chromosomes can facilitate the accumulation of sex-linked barrier traits, such as mating signals and preferences, whose associations might otherwise be broken down by recombination (Trickett & Butlin, 1994; Reinhold, 1998; Ritchie & Phillips, 1998; Sæther et al., 2007; Qvarnström & Bailey, 2009).

Two of the most consistent patterns seen in reproductive isolation also implicate sex chromosomes in establishing species boundaries: Haldane's rule (Haldane, 1922) and the large X effect, or disproportionate influence of X-loci on hybrid incompatibilities (Coyne & Orr, 1989; Masly & Presgraves, 2007; Presgraves, 2008; Schilthuizen et al., 2011). Studies of hybridisation have shown that X-loci tend to exhibit greater than expected differentiation (given the differences in Ne, recombination and linkage disequilibrium) and lower rates of introgression compared to autosomes, which is consistent with an overrepresentation of both pre- and postzygotic barriers on the X (Saetre et al., 2003; Payseur et al., 2004; Janoušek et al., 2012; Sankararaman et al., 2014; Larson et al., 2014; Lavretsky et al., 2015; Maroja et al., 2015; Cahill et al., 2015). Intraspecific studies have also shown that X chromosomes exhibit higher rates of substitutions (Betancourt & Presgraves, 2002; Mank et al., 2007; Mank et al., 2010; Meisel & Connallon, 2013; Garrigan et al., 2014), reduced diversity (Andolfatto 2001; Kauer et al., 2002; Baines & Harr, 2007; Begun et al., 2007; with the exception of African populations of *Drosophila melanogaster* and *D. simulans*) and greater population differentiation (Ford & Aquardo, 1996; Keinan et al., 2009; Amato et al., 2009; Lucotte et al., 2016; Machado et al., 2016). For example, Ford & Aquadro (1996) reported consistently higher differentiation at X-loci than at autosomal loci (up to 3.9x) in comparisons of pairs of semi-species of *Drosophila athabasca*.

Patterns of discordance among X and autosomal markers can be used to infer evolutionary processes that shape genomic variation, such as drift, selection, mutation, migration and mating system, as these sometimes act in different ways on sex chromosomes and autosomes (Charlesworth, 2001; Vicoso & Charlesworth, 2006; Ellegren, 2009; Emery et al., 2010). For example, relative levels of X/A diversity can be compared with predictions arising from demography. Under neutral expectations (1:1 breeding sex ratio and no selection), X chromosomes are expected to exhibit lower diversity (the ratio of X-to-autosomal diversity (X/A) is expected to equal 0.75) and increased population differentiation due to their smaller Ne (Vicoso & Charlesworth, 2006). Deviations from the expected level of diversity can indicate selection, demographic or sex-biased processes that shape genome variation (Charlesworth, 2001; Ramachandran et al., 2004; Hammer et al., 2008; Ségurel et al., 2008; Ellegren, 2009; Keinan et al., 2009; Emery et al., 2010) or variation in effective recombination rates (Vicoso & Charlesworth, 2009). For example, historic population contractions and expansions are expected to differentially affect X-to-autosomal diversity (X/A) (Pool & Nielsen, 2007); recent population bottlenecks tend to disproportionately reduce X-linked diversity while population expansions disproportionately elevate X-linked diversity (Schaffner, 2004). Geographic patterns of genetic diversity can also inform the processes involved in shaping genome variation (Hewitt, 2001; Sousa & Hey, 2013; Lexer et al., 2013). The extent to which individuals or populations deviate from the expected association between geographic and genetic distance (isolation by distance, IBD) enables inference about processes such as migration, admixture or selection (Wright, 1943, 1946; Wang et al., 2012; Fields et al., 2015; Knowles et al., 2016). Demography and selection are likely to influence autosomes and X chromosomes differently, resulting in distinct geographic

patterns of variation (Laurent et al., 2016). For example, X chromosomes might exhibit greater differentiation in sympatry due to a greater role in reproductive isolation (Martin et al., 2013).

Here, we compare the geographic distribution of genome-wide variation at autosomal and X-loci among populations of two closely related field cricket species, *Teleogryllus commodus* and *T. oceanicus*. These species are a classic system for sexual selection studies (Hoy & Paul, 1973; Hoy et al., 1977; Hennig & Weber, 1997; Bailey & Macleod, 2013), are partially interfertile (Moran et al., 2017) and overlap across a broad area of sympatry on the eastern coast of Australia (Hill et al., 1972; Otte & Alexander, 1983). Laboratory studies have found both pre-mating and post-mating barriers exist between them (Fontana & Hogan, 1969; Hennig & Weber, 1997; Moran, 2017), most notably hybrid females are sterile in both cross directions which provides a rare exception to Haldane's rule (Moran et al., 2017). Nevertheless, hybrid males mate successfully with females of both species, so while F1 hybrids are expected to be rare in sympatry, backcrossing and introgression might be expected. These species have an XO sex determination system, and the X chromosome appears to account for a substantial portion of their genome, (~20% - 30% of the genome (K Klappert; unpublished data/pers. comm.)).

There have been no previous genome-scale analyses of these Australian species (Pascoal et al., 2014 examined Hawaiian populations of *T. oceanicus*). Here, we used RADseq to compare patterns of genetic variation seen in putative X-linked and autosomal markers, and tested whether differentiation at X-loci was consistently greater both within and between species. As there is no published reference genome for this species, we focused on identifying X-loci using a series of stringent bioinformatic filters; this produced a smaller number of X-loci that would be predicted

given the predicted size of the X, but enabled us to produce a very high confidence set of X-loci.

We focused on testing two predictions: **(i)** Within population diversity ($\boldsymbol{\pi_S}$) should be lower at X compared to autosomal loci due to increased selection (exposure of beneficial recessive mutations), drift (smaller Ne compared with autosomes) and systematic variation in the opportunity for recombination (reviewed in Schaffner et al., 2004, Vicoso & Charlesworth, 2006, Ellegren et al., 2009; Mank et al., 2010). This would be expected to lead to greater population differentiation (F_{ST}) at X-loci. However, an alternative result is that faster-X dynamics might drive lower population divergence on the X if adaptive mutations are globally beneficial and spread readily across populations, despite also leading to reduced X-diversity (Nolte et al., 2013). We also tested whether species differentiation at X-loci differs between sympatric and allopatric comparisons. **(ii)** If demographic processes, such as population size changes, contributed to deviations from expected levels of X/A diversity then there should be an association between the demographic history of the populations and the observed level of X/A diversity. Due to its smaller Ne, the X is more sensitive to changes in population size such as occur during population bottlenecks or rapid expansions (Pool & Nielsen, 2007). Population contractions tend to reduce X diversity, whereas population expansions equalize levels of X/A diversity (Hammer et al., 2008). We therefore tested whether changes in population size contribute to observed levels of X/A diversity. The demographic history of the species can be inferred based on the site frequency spectrum and coalescent methods such as those implemented in Fastsimcoal (Excoffier et al., 2013). We report strong support for our prediction that interspecific divergence at X-loci is substantially higher than at autosomal loci. However, this pattern was unexpectedly

reversed in intraspecific population comparisons and X/A diversity was inflated for both *T. commodus* and *T. oceanicus*.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Individual crickets were sampled from 16 sites between March and April, 2013. Sampling encompassed a ca. 2,500 km latitudinal transect along the eastern coastal region of Australia and included allopatric and sympatric populations of both *T. commodus* and *T. oceanicus* (Figure 1). Areas of sympatry were located with guidance from published studies (Hill et al., 1972; Otte & Alexander, 1983; Bailey et al., 2017). Males and females were sampled from each population and preserved in methylated spirits followed by absolute ethanol. Sample sizes were approximately 30 individuals per population, except for “KH” and “UQ” where only 14 and 15 individuals were collected, respectively. Sample sizes and locality information for each population are provided in Table S1, Supporting Information. In the most northern population sampled (KH), we identified a third putative *Teleogryllus* species, *T. marini*, which has only previously been described once based on its distinct calling song and genitalia (Otte & Alexander, 1983). We disregarded putative *T. marini* samples when comparing patterns of genetic differentiation for X vs. autosomal markers due to a lack of adequate sampling, but report genomic population clustering results to definitively confirm its distinct species identity (see Results).

2.2 | Genomic library preparation

Genomic DNA was individually extracted from tissue removed from cricket head capsules (n=480, Table S1) with the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions for animal tissue. The extracted DNA was subsequently quantified and quality checked using Nanodrop and Qubit. RAD library preparation was carried out following Baird et al. (2008), with some modifications. Briefly, 250 ng of each DNA sample was digested with SbfI (New England BioLabs) and each individual cricket was barcoded by ligating P1 adapters (8 nM). Fragments were sonically sheared and size-selected to 300–700 bp and P2 adapters were ligated to sheared ends. Libraries were PCR amplified and paired end sequencing was conducted on 3 lanes of Illumina HiSeq 2000 and 2500 each. This protocol provides two sets of reads at each RAD site: read 1 extends either side of the restriction site (~ 100bp), while read 2 sequences are more loosely distributed extending up to ~700 bp from the restriction site (Davey et al., 2013).

2.3 | *De novo* assembly and SNP calling

The sequences were analysed using the Stacks RAD pipeline (Catchen et al., 2013) and following the RADmapper approach [https://github.com/tcezard/RADmapper]. Individual samples were demultiplexed with *process_radtags* from Stacks, allowing one mismatch in the restriction enzyme recognition site. Read 1 sequences from each sample were individually clustered using ustacks (parameters –M 2 –N 4). The resulting stacks were merged across samples within each species separately, and for all samples together, using cstacks with default parameters (Catchen et al., 2013).

The effect of assembly protocol and variant filtering is an important consideration for comparing divergent non-model species using RADseq (Lexer et al., 2013; Nadeau et al., 2014). Therefore, three different *de novo* assembly approaches were implemented to determine the most appropriate method for creating a consensus reference for calling single nucleotide polymorphisms (SNPs) that would enable us to obtain a maximum number of SNPs with minimal effects of ascertainment bias. The three different assemblies were constructed as follows: (i) a combined species assembly used individuals randomly subsampled from all populations and pooled together, (ii) a *T. commodus* assembly used pooled allopatric *T. commodus* individuals, and (iii) a *T. oceanicus* assembly used pooled allopatric *T. oceanicus* individuals. The final merged stacks were filtered to remove potential erroneous stacks by retaining only those supported by a minimum number of individuals (combined species assembly, n = 200; *T. commodus* assembly, n = 100; *T. oceanicus* assembly, n = 50).

Read 1 sequences of all individual samples were mapped back against the consensus sequences using BWA v0.7.9a (aln/samse algorithm) (Li & Durbin, 2009). The mapping statistics were generated using *samtools flagstat*. To extend the usable sequence for SNP calling, the read 2 sequences for each read 1 stack were assembled using the IDBA-UD v1.1.0 assembler following the methods of Peng et al. (2012). The resulting read 2 assembly was then merged with the read 1 stack where possible, otherwise the sequences were concatenated. Variants were called using SAMtools/BCFtools (v0.1.18) (Li et al., 2009; Li, 2011) and SNPs outputted in Variant Called Format (VCF) files.

To assess if the different *de novo* assemblies influenced the downstream analysis, we compared the number of SNPs obtained from each and the overlap among them. The three different assemblies resulted in a similar number of SNPs and the structuring of population genetic variation was broadly the same, with no sign of species bias. Detailed comparisons of the number of SNPs returned from the three different assemblies is provided in Figure S1, Supporting Information. Therefore, we proceeded using the combined species assembly to address patterns of interspecific genotypic variation, whereas we used species-specific assemblies for intraspecific analyses to maximize the number of potentially informative markers.

2.4 | SNP quality control and genetic diversity estimates

VCF files were filtered using VCFtools (Danecek et al., 2011) using the following steps: all indels were removed, only sites with a minor allele frequency greater than or equal to 0.05 were kept, and all individual genotypes with a quality score less than 20 were recoded as missing (*--min GQ* 20 – VCFtools command). Next, all loci that were not present in at least 80% of the individuals were excluded using the *--max-missing* VCFtools command. Fifteen individuals were dropped due to low mapping coverage or a high proportion of missing genotypes (< 50 % mapped reads or genotypes present). For population genetic analysis, the quality filtered VCF files which contained only biallelic SNPs were converted to required file types using PGDSpider (Lischer & Excoffier, 2012). The number of SNPs varied in some analyses due to subsetting of the data, which changes the proportion of missing data.

2.5 | Putative autosomal and X-loci

A set of high-confidence, putative autosomal and X-loci were identified using custom scripts to filter markers based on expected sex differences in heterozygosity and read coverage. *Teleogryllus* spp. females carry two X chromosomes while males only carry one, so X-loci should have twice the read depth in females and should appear homozygous in males. The following criteria were applied to filter X SNPs. Criterion 1: For a given locus, all male samples must be homozygous and at least one female sample must be heterozygous. Criterion 2: To filter based on coverage, first the overall coverage was normalised by the total number of reads in each sample (to minimise artefactual heterogeneity in read depth introduced by variation among libraries or sequencing lanes), and then male vs. female samples were compared using a Student's t-test. After adjusting for multiple testing (Bonferroni correction) candidates with p-values < 0.05 were retained (where coverage was lower in males compared to females). Criterion 3: Finally, the mean fold change in coverage was calculated between male and female samples, and only candidate SNPs within the range of 1.8 – 2.2 fold change were selected.

A similarly stringent set of filtering criteria were implemented to filter autosomal loci. Criterion 1: For a given locus, at least one male and one female sample must be heterozygous. Criterion 2: First normalize the overall level of coverage as before and select candidates which show no significant difference in coverage between males and females (Student's t-test: select SNPs with p-values > 0.05). Criterion 3: Only candidate SNPs within the mean fold change range of 0.8 – 1.2 were selected.

Overall, this yielded: n = 2,405 X SNPs and n = 26,447 A SNPs using the *T. commodus* assembly, n = 1,288 X SNPs and n = 34,010 A SNPs using the *T. oceanicus* assembly, and n = 1,838 X SNPs and 23,411 A SNPs using the combined assembly. The relatively low number of X-linked SNPs obtained (~4 – 9% of the overall SNPs) might be due to our conservative approach to filtering markers (putative X SNPs must pass all three filtering criteria), or a lower X diversity, which results in a small but high confidence set of X-loci . Loci that failed to be assigned to either the X or autosomal groups were omitted from further analyses ensuring a high confidence in the distinction between X and autosomal marker groups (Figure S2). To ensure our results were robust to the filtering procedure we varied the filtering thresholds by increasing the fold-change range and removing the p-value check (Table S3). To visualize the distinction between the X and A SNPs retrieved we plotted the fold-change and heterozygosity (males vs. females) for each SNP (Figure S2). We compared the results of the different filtering criteria based on the number of SNPs retrieved and downstream summary statistics such as F_{ST} and nucleotide diversity (Tables S4-6). Overall the results were highly consistent between the different filtering approaches.

2.6 | Population genetic structure

We used a combination of approaches to test for introgressive hybridization and to characterise the pattern of genotypic variation within and between species. The ancestries of individuals were examined using FastStructure (Raj et al., 2014), which applies a Bayesian method to assign individuals to genetic clusters and estimate an admixture proportion for each individual. We first ran the analysis on the total dataset

(combined assembly) and then subsetted the data into species-specific groups to explore how genetic variation is distributed within each species. The analysis was run for K = 1-10 groups using simple priors (i.e. a flat beta-prior over population specific allele frequencies for each SNP) (Raj et al., 2014). The most likely number of groups (K) was assessed with the "chooseK.py" script from FastStructure, which identifies the range of K values that provide the lowest model complexity while maximizing the marginal likelihood and the minimum number of components needed to explain structure in the data. To detect fine-scale genetic structuring of populations, the analysis was rerun using logistic priors (i.e. a logistic normal distribution for each SNP used to estimate the population-specific allele frequency), based on the previously obtained most likely value of K. Multiple replicates (30 - 50) were run and the average from the top 25 most likely replicates was used. Admixture proportions were plotted using the "distruct.py" script. To further explore population genetic structure, principal component analysis (PCA) was applied using the R packages *SNPRelate* (Zheng et al., 2012) and *Adegenet* (Jombart et al., 2010). Individual scores were plotted to visualize the distribution of genetic diversity across populations, and the genetic relationships among populations of both species were visualized using hierarchical clustering trees for pairwise F_{ST} values.

2.7 | Comparison of genetic variation at autosomal and X-linked markers

Genetic summary statistics were estimated for each population, including observed heterozygosity (H_{obs}), expected heterozygosity (H_s), and the inbreeding coefficient (F_{IS}), using the R packages *Adegenet* (Jombart et al., 2011; Jombart, 2008) and *Hierfstat* (Goudet, 2005). Nucleotide diversity (π) was calculated per-site (SNP)

using VCFtools (--site-pi) (Danecek et al., 2011). This measure represents an approximation based on variant sites only. To account for sequence length including invariant sites, we standardized π per-population and per-species by overall SNP density from each of the four combinations of species and chromosome type (π per-site/ (total sites/variable sites)) (Zhang et al., 2016). We report the standardized measure of π in our Results and Discussion although both measures resulted in very similar levels of X/A diversity (Table S7). Total diversity (π_T) was partitioned into within-population diversity (π_S) and between-population diversity (π_B) ($\pi_B = \pi_T - \pi_S$; Charlesworth, 1998). Genetic summary statistics for X and autosomal-loci estimates were based on female only datasets to avoid the influence of male hemizygosity. Pairwise population F_{ST} values were calculated using *Hierfstat* (Goudet, 2005), following the method of Weir & Cockerham, (1984). Significance of population comparisons was tested with 1,000 bootstraps (using the boot.ppfst function), and significance was inferred if the confidence intervals did not overlap zero. To test whether autosomal and X-loci differed in the pattern of differentiation we used one-tailed Wilcoxon sum rank tests. We examined the relationship between population diversity (intra-population (π_S) and total population diversity (π_T)) and F_{ST} using an equation originally proposed by Hudson et al., (1992; eq. 3: $F_{ST} = 1 - \pi_S / \pi_T$).

Estimates of linkage disequilibrium (LD) were obtained using the R package *Genetics* (Warnes et al., 2013). Only SNPs that passed the following quality criteria (applied using VCFtools) were retained for LD estimation; only a single SNP per locus, no missing genotypes, minor allele frequency > 0.1 and an overall genotype quality score >20. This resulted in 437 X SNPs for *T. oceanicus* and 836 X SNPs for *T. commodus*. A large number of A SNPs passed the quality filtering for both species

but in order to reduce the number of pairwise comparisons a random subset of 1,000 SNPs were selected from the quality filtered set.

We tested whether discordance between marker types could be explained by differences in the relative effective population size of the X by calculating expected values of F_{ST} at X markers using the formulas proposed by Ramachandran et al., (2004) and Ségurel et al., (2008). These formulas allow one to predict the expected level of differentiation at X-loci based on the observed autosomal values, given a particular breeding sex ratio and migration rate, according to Suppl. Eq. 1 & 2 (Ségurel et al., 2008; Machado et al., 2015). Suppl. Eq. 1 & 2 and associated formulas are described in the Supplemental Formulas section of the Supporting Information. We checked whether pairwise F_{ST} values for autosomal loci were correlated with those for X-loci, using a Mantel test implemented in the R package *ade4* (Dray & Dufour, 2007). Significance was based on 1,000 permutations. Sex-biased dispersal may also alter the relative Ne of the X compared to autosomes, so we tested for this by comparing the average F_{ST} values at autosomal loci between males and females for both species (however this is a conservative approach as sex-biased dispersal would need to be strong and consistent to be detected using this method).

2.8 | Population demographic history

To examine the demographic history of populations we used a composite likelihood approach implemented in Fastsimcoal2 (Excoffier and Foll, 2011). Fastsimcoal uses a coalescent-based approach to obtain the expected site frequency spectrum (SFS) under different demographic models and estimates the parameter combinations that

maximize the composite likelihood (Excoffier et al., 2013). In the absence of information on the derived allele state the minor allele frequency was used to calculate the folded SFS using a custom R script (available here: https://github.com/shenglin-liu/vcf2sfs). To increase the numbers of SNPs for better model fitting we examined single populations instead of joint populations. In addition, we selected 10 females per population which had the lowest amount of missing data. Only SNPs that passed the following quality criteria (applied using VCFtools) were retained for constructing the observed SFS; only a single SNP per locus, no missing genotypes, minor allele frequency > 0.05 and an overall genotype quality score >20. The number of monomorphic sites was calculated based on the total sequence lengths (reads which contained the quality filtered SNPs) minus the number of polymorphic sites (SNPs and linked sites which were removed prior to this analysis). After quality filtering we retained ~14,000 – 21,000 SNPs per population.

Four different demographic models were compared for populations of both species: a simple model of a population of constant size, a second model of exponential growth, a third model of population contraction and a fourth model of a population bottleneck. For each model we conducted, 50 independent runs per population and 100,000 simulations (40 ECM cycles and a stopping criteria of 0.001) for maximum likelihood estimation. We used a mutation rate of 3.5×10^{-9} per generation (Keightley et al., 2009). The run with the highest maximum likelihood was used for parameter estimation and model comparisons. To test the sensitivity of our results to the mutation rate, we also estimated parameters based on a mutation rate of 2.5×10^{-8} for one of our study populations (AM). As expected this resulted in parameter estimates which differed in scale but most importantly the relative level of

population size change (NPOP/NANC) remained the same. To decide which demographic model fitted our data best we used AIC model comparisons and Akaike weights. 95% confidence intervals were estimated from 100 parametric bootstraps (20 runs and 100,000 simulations per simulated site frequency dataset).Our primary focus was on model comparison rather than parameter estimation as the absolute values for the latter may be sensitive to uncertainties such as the mutation rate.

To test whether population size changes can account for the level of X/A diversity we calculated expected X/A diversity given a population size change using a formula proposed by Pool & Nielsen (2007) (Suppl. Eq. 3). The demographic parameter estimates (e.g. current effective population size, population size prior to the size change and the time in generations ago the size change occurred) were obtained from the models with highest likelihoods from Fastsimcoal. Under neutral assumptions the effective population size of the X is 0.75 and for autosomes is 1. Applying Suppl. Eq. 3 and adjusting the inheritance factor (h) we examined how changes in both the effective population size of these genomic regions and population size changes jointly impact X/A diversity.

2.9 | Geographic distribution of genetic variation

We examined patterns of IBD at either X or autosomal loci by testing the relationship between pairwise population F_{ST} values and Euclidean geographic distances (estimated from each site's latitude and longitude coordinates obtained from Google maps (https://www.google.co.uk/maps)) using Mantel tests in *ade4* (Dray & Dufour, 2007) using 10,000 permutations.

T. commodus and *T. oceanicus* could differ in their relationship with geographic distance and genetic divergence, i.e. patterns of IBD vary between species, so to test this we estimated and compared the frequency distribution of 1,000 slopes and intercepts at autosomal and X-loci using the R package *boot* (Canty & Ripley, 2008; following the methods of Baselga, 2010). The probability of one species having a higher slope or intercept than the other, at autosomal versus X-loci, was estimated by examining the proportion of bootstraps for which one species had higher parameters than the other. To obtain *p*-values, we used the probability of obtaining the opposite result by chance by comparing the estimated distribution of parameters (Baselga, 2010).

3 | RESULTS

3.1 | Population genetic structure and the species boundary

FastStructure analysis on the total dataset (16 populations; combined species assembly) revealed four genetic groups corresponding to three putative species and a north-south latitudinal gradient among *T. commodus* populations (Figure 2A). There was no evidence for recent species admixture among either *T. commodus* and *T. oceanicus* or *T. oceanicus* and *T. marini*, with a clear bimodal distribution of pure species individuals in sympatry (admixture proportions either > 0.9 or < 0.01) (Figure 2B). An admixture proportion of ca. 0.5 would indicate F1 hybrids, while >0.25 and <0.75 would be classified as recent backcrosses (Pritchard et al., 2000; Raj et al., 2014). Because sympatric individuals were clearly assigned to either *T. commodus* or *T. oceanicus* species groups, we subsetted the data into species-specific groups to further examine intraspecific population genetic structure. Intraspecific analyses

revealed that for both species, the most likely number of groups ranged between 2 – 3 (Figure 2.C). *T. commodus* populations exhibited a clear latitudinal gradient of genetic variation distinguishing southern from northern populations. In contrast, there was no clear geographic structuring among *T. oceanicus populations*, with individuals from multiple populations sharing similar admixture proportions (one exception being the DV population; Figure 2.C).

Principal components analysis clearly differentiated genotypic variation among the species along the first principal component (PC), which explained 18% of the variation in the data (Figure 3A). Individuals of the putative species *T. marini* clustered separately from the two other species. PC2 distinguished southern and northern populations of *T. commodus*, but accounted for only 1.18% of the variation. Sympatric individuals were clearly placed within the two main clusters, and there was no evidence of intermediates. In separate, species-specific analyses, PC1 and PC2 accounted for far less genotypic variation (1-3%). Among *T. commodus* populations, PC1 distinguished southern from northern populations (Figure 3B), but *T. oceanicus* was less structured and showed considerable overlap among allopatric and sympatric populations (Figure 3C).

3.2 | Inflated X/A diversity

Nucleotide diversity (π_S) was slightly higher at X-loci compared to autosomal loci for *T. commodus* (X/A: 1.084 [95% CI: 1.069 – 1.099]; two-tailed Wilcoxon sum rank test $W = 118$, $p = 0.0002$) but lower at X-loci within *T. oceanicus* populations (X/A: 0.923 [95% CI: 0.902 – 0.944; $W = 6$, $p = 0.007$) (Figure 4.i). Nevertheless, the ratio of X-to-autosomal diversity (X/A) was significantly greater than the expected value of 0.75

(under neutral assumptions) for both species (*T. commodus* one-tailed Wilcoxon test, V = 66, $p < 0.001$; *T. oceanicus :* V = 36, $p = 0.004$). There was a non-significant trend for between-population diversity (π_B) to be lower at X-loci within both species (Table 1) (*T. commodus*: two-tailed Wilcoxon sum rank test; $W = 80$, $p = 0.211$; *T. oceanicus*: $W = 39$, $p = 0.495$), possibly due to the overall low level of between-population diversity which is bounded at 0, and the large variance in the estimates. There was also a non-significant trend for the inbreeding coefficient (F_{IS}) to be higher at X-loci among both species (*T. commodus*: two-tailed Wilcoxon sum rank test; $W = 55.5$, $p = 0.768$; *T. oceanicus*: $W = 26$, $p = 0.574$) (Table 1). Estimates of LD (r2 and D') were very low for both species at X and A markers and there was no clear difference between marker types (Table S9). Similarly, there was no obvious difference in the site frequency spectrum (folded) for X and A markers in populations from both species groups (Figure S7).

3.3 | Population growth does not account for inflated X/A diversity

There was a clear signal of population growth across the broad range of populations sampled (Table 3, S10). A model of exponential growth fitted best compared to models of constant population size, contraction or bottleneck, with most of the relative AIC weight assigned to the model of population growth (Table S10). Although there was not a large difference in support for the bottleneck and population expansion model (Delta AIC < 2), parameter estimates from the bottleneck model were consistent with those of the expansion model, indicating a similar level and timescale of population growth (Table S11).

The extent of population growth was not sufficient to account for the inflated X/A diversity (Figure 5). Adjusting the inheritance factor (h) (Suppl. eq. 3) to reflect an increase in the effective population size of the X revealed that sex-biased processes could have had a greater impact on X/A diversity (Figure 5). However, for *T. commodus* populations even after accounting for population size changes and an increased effective population size of the X, the observed X/A diversity was still higher than expected (X/A observed vs. X/A expected (given the pop size change and adjusted X inheritance factor of 0.95): two-tailed Wilcoxon sum rank test W =16, p =0.021). Demographic parameter estimates were roughly similar across populations suggesting a similar timing and rate of population growth (Table 3).

3.4 | Intraspecific differentiation is lower for X-loci

Contrary to our prediction that X-loci would show more pronounced population genetic structuring compared to autosomal loci, there was a striking reduction in differentiation at X-loci among populations within each species (Figures 4.iii). The discordance between autosomal and X-linked markers was particularly pronounced among *T. oceanicus* populations, with all pairwise population comparisons showing reduced F_{ST} values at X-loci (one-tailed Wilcoxon sum rank test: H_0: F_{ST} (A) = F_{ST} (X), H_1 F_{ST} (A) > F_{ST} (X), W = 711.5, p < 0.001) (Figure S3). For autosomal loci, nearly all *T. oceanicus* populations were differentiated (albeit weakly) (average pairwise population F_{ST} = 0.018 [95% CI: 0.017 - 0.02]). In contrast, there was very little population structuring at X-loci (F_{ST} = 0.001 [-0.002 - 0.005]), with most pairwise population comparisons not significant (95% CIs overlapping with 0). Genetic differentiation was also lower at X-loci among *T. commodus* populations (F_{ST} (A) =

0.036 [0.034 - 0.037] vs. F_{ST} (X) = 0.018 [0.013 - 0.023]) (one-tailed Wilcoxon sum rank test: W = 2057, p < 0.001).

The observed level of differentiation at X-loci was much lower than that expected under an equal sex ratio (r = 0.5) in both *T. commodus* (0.046 [95% CI: 0.037 – 0.056]) (one-tailed Wilcoxon sum rank test: W = 2223; p < 0.001) and *T. oceanicus* (0.024 [0.016 – 0.032]) (W = 727; p < 0.001) (Figure 6). Sex-biased processes that increase the effective population size (Ne) of females (and therefore X chromosomes) should lead to reduced differentiation at X-loci, bringing it closer in line with the autosomes (Figure 6). However, even under an extreme female sex-ratio bias (e.g. r = 0.9) and a strong female biased migration rate (e.g. mf/m = 0.9) expected differentiation at X-loci (*T. commodus*: 0.028 [95% CI: 0.022 – 0.034]; *T. oceanicus* : 0.014 [0.009 – 0.019]) is still significantly greater than our observed level in both species (*T. commodus*: one-tailed Wilcoxon sum rank test: W = 1891; p =0.012; *T. oceanicus* : W = 687, p < 0.001). Similarly, examining the relationship between population diversity (π_S and π_T) and F_{ST} using equation 3 from Hudson et al., (1992), the expected F_{ST} was also higher than that observed (Figure S4). Other factors may contribute to the discrepancy between the observed and expected level of differentiation such as the simplified assumptions of these models (based on Wrights infinite island model (Ségurel et al., 2008)) which strong isolation by distance (which we observe in this system) may violate. In addition we found no evidence to support strong sex-biased dispersal in either species as there was no difference in the average F_{ST} values at autosomal loci among males and females (Wilcoxon sum rank test: *T. oceanicus, W* = 398.5, p = 0.922; *T. commodus W* = 1570.5, p = 0.731) (however, this method is conservative as sex-biased dispersal would need to be strong and consistent for its signal to be detected using this approach).

Within each species, the strength of isolation by distance (IBD) differed between marker types (Figure 7, Tables 2, S9). However, this marker-associated difference in IBD was of greater magnitude in *T. oceanicus* populations than in *T. commodus* populations (Figure 7, Table 2). Within both species, IBD was stronger at autosomal loci compared to X-loci. There was a particularly strong reduction in differentiation among *T. oceanicus* populations for X-linked compared to autosomal loci, with significant positive IBD at autosomal loci (Mantel: r = 0.562, p = 0.016) but a weakly negative, non-significant association at X-loci (Mantel: r = -0.378, p = 0.973). In contrast, genetic differentiation for both marker types among *T. commodus* populations exhibited a positive relationship with geographic distance, but while IBD was very strong for autosomal loci (Mantel test: r =0.916, p <0.001), it was weaker for X-loci (r = 0.495, p = 0.005).

3.5 | Interspecific differentiation is higher for X-loci

In line with our main prediction, there was stronger differentiation between *T. commodus* and *T. oceanicus* at X-loci than at autosomal loci (Figures 4.vi, 6). Interspecific population comparisons resulted in an average pairwise population F_{ST} of 0.331 (95% CI: 0.324 – 0.336) at autosomal loci in contrast to 0.484 (0.461 – 0.507) at X-loci (one-tailed Wilcoxon sum rank test: H_0: F_{ST} (A) = F_{ST} (X), H_1 F_{ST} (A) < F_{ST} (X), W = 7744, p < 0.001). Species differentiation at X-loci was greater than expected even after accounting for the reduced effective population size of the X (assuming an equal sex ratio by assigning r = 0.5 in Suppl. eq.1: expected F_{ST} (X): 0.397 [0.394 – 0.4]) (X observed vs. X expected: W = 7734, p < 0.001).

The potential for species interactions between *T. commodus* and *T. oceanicus* appeared to influence X-linked differentiation: there was greater interspecific differentiation at X-loci in sympatry (F_{ST}: 0.523 [95% CI: 0.499 – 0.546]) than in allopatry (0.468 [0.445 – 0.490]) (one-tailed Wilcoxon sum rank test: $W = 378$, $p < 0.001$) (Figure 4.vi). However, the sample sizes in sympatry, in particular for *T. commodus*, were relatively small so caution is advised when interpreting these differences.

The greater differentiation between the species at X- compared to autosomal-loci appears to be driven at least partially by a reduction in X diversity, particularly within *T. oceanicus* (Figure 4.iv). Nucleotide diversity (π) was substantially reduced at X-loci within *T. oceanicus* populations X/A: 0.634 [95% CI: 0.621 – 0.648]; two-tailed Wilcoxon sum rank test $W = 64$, $p < 0.001$) but higher at X for *T. commodus* (X/A: 1.244 [95% CI: 1.226 – 1.262]; two-tailed Wilcoxon sum rank test $W = 121$, $p < 0.001$) when estimates were based on the combined species approach (see Methods 2.3). Importantly, the extent of the reduction in X diversity appears to be sensitive to the filtering method used (Figure 4.i - iv). In particular, X/A diversity among *T. oceanicus* populations was much lower when the SNPs were retrieved from the combined species pool of individuals (X/A: 0.634 [0.621 – 0.648]) (Figure 4.iv), than when estimates were based on SNPs ascertained from only *T. oceanicus* individuals (0.923 [0.902 – 0.944]) (Figure 4.i). We elaborate on this potential ascertainment bias further in the Discussion.

4 | DISCUSSION

4.1 | Opposing patterns of differentiation on the X at different scales

Geographic areas where interfertile species overlap provide important testing grounds for determining how porous species boundaries are, and for identifying the main barriers to gene flow (Payseur & Rieseberg, 2016). We investigated genetic structure at autosomal and X-loci in two closely related field cricket species, *T. commodus* and *T. oceanicus,* to test predictions about the consistency of patterns in intra- versus inter-specific comparisons. Theory predicts reduced genetic diversity on X chromosomes and increased differentiation within and between species relative to autosomes, due to differences in the efficacy of selection and drift on these regions (Charlesworth et al., 1987; Betancourt et al., 2004; Vicoso & Charlesworth, 2006; Mank et al., 2010). In line with this prediction, we found greater species differentiation at X-loci even after accounting for their reduced effective population size (Figures 6). However, within species comparisons revealed an unexpected and striking pattern of reduced population differentiation at X-loci and higher than expected X-to-autosomal diversity (Figures 4, 5).

The results from our intraspecific comparisons are counterintuitive and contradict most previous studies, which reported reduced diversity and higher levels of genetic differentiation for X-loci, both within and between species, across a range of taxa including humans (Ramachandran et al., 2004; Keinan et al., 2009; Amato et al., 2009; Lucotte et al., 2016), birds (Borge et al,, 2005; Ellegren et al., 2012; Ruegg et al., 2014; Lavretsky et al., 2015), butterflies (Martin et al., 2013), rabbits (Carneiro et al., 2010), and *Drosophila* (Ford & Aquadro, 1996; Andolfatto, 2001; Machado et al., 2016). However, there are some exceptions in which populations have been

found to exhibit higher levels of genetic differentiation at autosomal loci, and in which X/A diversity is greater than expected, notably in African populations of humans (Ségurel et al., 2008; Hammer et al., 2008) and *Drosophila* (Andolfatto, 2001; Nolte et al., 2013; Machado et al., 2016; Sedghifar et al., 2016). The magnitude of discordance in the level of differentiation at X and autosomal markers in our study, in particular in *T. oceanicus,* is much greater than the exceptions listed above.

An important consideration is whether our use of F_{ST} as a measure of divergence could have confounding effects on estimates of divergence, as it is a relative measure and thus dependent on the proportion of within- and between-population variation (Charlesworth, 1998; Jakobsson et al. 2013; Cruickshank & Hahn, 2014). Low levels of within-population diversity could inflate F_{ST}, whereas high diversity could reduce F_{ST} (Cruickshank & Hahn, 2014). We therefore considered whether the reduced differentiation among populations within species at X-loci could be due to higher than expected X diversity within populations? In *T. commodus*, intra-population diversity was slightly higher at X-loci compared to autosomal loci (X/A: 1.084 [95% CI: 1.069 – 1.099]). This higher diversity is likely to contribute to the reduced differentiation at X-loci, which was 2 times lower than at autosomal loci (mean F_{ST} X: 0.018 [0.013 – 0.022], A: 0.036 [0.034 – 0.037]) while between population diversity was 1.6 times lower (mean π_B X: 0.142×10^{-4}, A: 0.232×10^{-4}). Therefore among *T. commodus* populations higher intra-population diversity could contribute to the reduced X differentiation. In contrast, nucleotide diversity within *T. oceanicus* populations, was lower at X-loci compared to autosomal loci (X/A: 0.922 [95% CI: 0.902 – 0.944]). While between-population X diversity was 5.2 times lower (X: 0.243×10^{-5}, A: 0.126×10^{-4}) and X differentiation was 18 times lower (X: 0.001 [-0.002 – 0.005], A: 0.018 [0.017 – 0.02]). As X diversity is lower within *T. oceanicus*

populations compared to autosomal loci the reduced X differentiation is unlikely to be solely an artefact of within population diversity. Overall, X/A diversity was inflated above neutral expectations in both species.

In contrast, for inerspecific comparisons, could the greater species differentiation at X-loci be an artefact of reduced X diversity? Analysis based on SNPs retrieved from the combined species pool revealed substantially lower intra-population diversity at X compared to autosomal loci for *T. oceanicus* ; (X/A: 0.634), but the opposite for *T. commodus* (X/A: 1.244) (Figure 4.iv). Between-species diversity was 1.6 times higher at X- compared to autosomal loci (mean π_B: X: 0.2 x 10^{-3} , A: 0.13 x 10^{-3}) while species differentiation was 1.46 times higher (mean X F_{ST}: 0.484 [95% CI: 0.459 – 0.508]; A F_{ST}:0.331 [0.324 – 0.336]) (Figure 4.vi). This suggests that the reduced X diversity within *T. oceanicus* populations drives the greater species differentiation at X-loci. We discuss below whether the reduced X diversity for *T. oceanicus* might reflect an ascertainment bias during SNP filtering.

Technical problems such as null alleles or paralogous loci are an important consideration in any RADseq study as they can potentially lead to biased diversity estimates (Gautier et al., 2013; Arnold et al., 2013). Null alleles may lead to an excess of homozygous loci as any individual that is heterozygous at a SNP associated with a null allele will be classified as a homozygote for the alternate successfully digested allele. A high level of null alleles is expected to bias diversity estimates downwards (heterozygous deficit), whereas paralogues are expected to inflate diversity estimates (heterozygous excess). In our study, removal of loci which significantly deviated from HWE did not affect our overall results or conclusions (Tables S3-4).

Another important consideration is the ascertainment of SNPs. As RADseq data can be highly variable in the amount of missing data, different sets of variants might be retrieved when SNPs are retrieved from the combined species pool of individuals (for the interspecific analysis; Figure 4.iv-vi) or from the smaller species specific groups (intraspecific analyses Figure 4.i-iii) due to filtering thresholds on the amount of missing data per SNP (in our analysis a valid SNP must be present in at least 80% of the individuals). The amount of missing data can influence the estimates of summary statistics such as π and theta (Gautier et al., 2013), although F_{ST} has been suggested to be more reliable (Arnold et al., 2013). In our study, there was a discrepancy in the estimate of X/A diversity, in particular among *T. oceanicus,* depending on whether SNPs were ascertained from the combined species group or the smaller species specific groups. X/A diversity among *T. oceanicus* populations was much lower when the SNPs were retrieved from the combined species pool of individuals (X/A: 0.634 [0.621 – 0.648]), than when estimates were based on SNPs ascertained from only *T. oceanicus* individuals (0.923 [0.902 – 0.944]) (Figure 4.i-iv). This suggests an ascertainment bias when retrieving X SNPs from the combined species pool, leading to an underestimation of X diversity among *T. oceanicus*. This bias is not due to the disproportionately greater number of samples from *T. commodus* compared to *T. oceanicus* (265 vs. 200) or the assembly method used (combined species assembly vs. species specific assemblies) (Table S12) but instead is due to the quality filtering criteria which removes variants that are not present in at least 80% of the individuals and that have a minor allele frequency greater than or equal to 0.05 (Figure S5). The fact that X diversity was disproportionately reduced in *T. oceanicus* in the combined species analysis supports the conclusion of greater species divergence at X-loci. Overall, SNPs that

were ascertained based on the species-specific groups (i.e. those used in the intraspecific analyses) are likely to provide more reliable estimates of within species X/A diversity. Analyses based on the species specific SNP datasets strongly support the conclusion that X/A diversity is inflated for both species.

4.2 | Evolutionary processes driving X/A patterns at different scales

What factors could contribute to the apparently contradictory pattern of greater differentiation between species but lower differentiation within species at X-loci? Greater species differentiation at X-loci, in particular among sympatric populations (Figure 4.vi), may reflect an important role for X chromosomes in reproductive isolation (Nachman & Payseur, 2012; Martin et al., 2013 but see Cruickshank & Hahn, 2014). This is supported by numerous hybrid zone studies which show greater differentiation and reduced introgression at X-loci (Payseur et al., 2004; Teeter et al., 2010; Carneiro et al., 2010; Maroja et al., 2015) possibly due to a greater role for X chromosomes in hybrid incompatibilities (Masly & Presgraves, 2007). However, in our study system, there was no evidence for recent gene flow between the species across a broad area of sympatry (Figure 2). Although small amounts of introgression or ancestral periods of species admixture may have occurred, these would require more in-depth demographic modelling to account for. The absence of recent hybridization suggests strong assortative mating (or fertilization) and/or habitat segregation (Jiggins & Mallet, 2000). A combination of both pre and post-mating barriers have been detected in *Teleogryllus* spp., including reduced courtship behaviour (Hill et al., 1972; Hennig & Weber, 1997; Bailey & Macleod, 2013; Moran, 2017) and hybrid female sterility (Moran et al., 2017). Stronger selection on the X in

sympatry possibly driven by species interactions (e.g. character displacement) might have contributed to the greater species differentiation at X-loci in sympatry.

Deviations from expected neutral patterns of diversity of sex and autosomal markers are usually interpreted as resulting from selection or demographic effects that alter the Ne of these genomic regions (Charlesworth, 2001; Vicoso & Charlesworth, 2006; Emery et al., 2010; Garrigan et al., 2014). For example, sex-biased processes, such as occur due to sex differences in adult mortality and fertility, can alter the relative effective population size of X and autosomal regions (Charlesworth, 2001). In *Teleogryllus*, a number of different processes acting together might have contributed to the higher than expected level of X/A diversity. In particular, strong sexual selection and a high variance in male reproductive success would increase the female effective population size, which could dramatically inflate the relative genetic diversity of X chromosomes (Figure 5) (Charlesworth, 2001; Hammer et al., 2008). In line with this, previous studies have shown that males of both *Teleogryllus* species mate multiply and there is a strong skew in reproductive success, with the bias being higher in *T. oceanicus* (Simmons & Beveridge, 2010). Sex-biased dispersal can also alter the relative Ne and genetic variation at X and autosomal loci. If females dispersed more frequently than males this could equalize the level of X/A divergence among populations and increase the relative genetic diversity of the X (Ellegren, 2009). However, we found no evidence for strong female-biased dispersal for either of the species (although, a sex-bias in dispersal would need to be strong and consistent to be detectable in comparisons between the sexes at autosomal loci). In addition, sex-biased dispersal is unlikely to uniformly influence X/A diversity across all populations (Laporte & Charlesworth 2002; Hammer et al., 2008). As the pattern of reduced differentiation at X loci is consistent

across populations (Figure 7), it suggests the driving factor is likely to be common across the species range rather than a localized environmentally induced effect.

Population size changes are expected to differentially affect the relative X/A diversity (Pool & Nielsen, 2007). Recent population bottlenecks are expected to lead to lower-than-expected levels of X/A diversity, whereas population expansions tend to equalize levels of X/A diversity (Hammer et al., 2008). Demographic models strongly supported population expansions across the range of both *Teleogryllus* species (Table S9) with roughly similar estimates for the timing and scale of population growth (Table 3). However, the magnitude of population size changes were not strong enough to account for the inflated X/A diversity we observed (Figure 5).

Both species are likely to have undergone cycles of inter-glacial contractions and expansions and more recent population shifts due to human disturbances (Chapple et al., 2011; Moritz et al., 2009; Singhal & Moritz, 2013), In the wet tropics of northeastern Australia, where we encountered the third putative species *T. marini*, an unusually high level of cryptic species diversity has been characterized, mainly in amphibians and reptiles, and appears to be due to the repeated sequestration of species ranges into mesic refugia during glacial periods, followed by population expansions during interglacials (Hilbert et al., 2007; Moritz et al., 2009; Singhal & Moritz, 2013). A previous study by Cairns et al., (2010) found evidence for a recent population expansion in a *T. commodus* population from New South Wales which they suggested was due to recent human disturbance and the expanse of agricultural land. The approximate estimated time of population expansion for our study populations is 37,500 generations ago, which predates recent human disturbance in Australia. However, caution is advised interpreting parameter

estimates as absolute values depend on the mutation rate which is unknown for our study species (using a mutation rate of 2.5 x 10-8 we obtain a time of expansion of ~4,900 years ago for AM population).

Finally, background selection (selection against deleterious mutations) is predicted to disproportionately reduce autosomal diversity as the autosomes contain a higher frequency of deleterious recessive mutations (Charlesworth, 1996; Betancourt et al., 2004). Such effects are generally expected not to be large, as the strength of background selection is dependent on rates of recombination, with the strongest effect in regions of low recombination (Charlesworth, 1996; Hammer et al., 2008). Autosomes are often expected to experience higher recombination than X chromosomes as male hemizygosity restricts X recombination to females. However, in many insect species, most notably *Drosophila* and Lepidoptera (reviewed in Wright et al., 2016; Stapley et al., 2017), but also in some Orthopterans (White 1965), recombination only occurs in the homogametic sex which would lead to more similar rates of recombination between X and autosomal regions (Betancourt et al., 2004). In cases where recombination is restricted to the homogametic sex linked-selection can lead to inflated X/A diversity (greater than 1 when variants under selection are dominant) (Betancourt et al., 2004). In our study species the lack of a clear difference in linkage disequilibrium between X and A loci suggests recombination rates may be similar between both marker types (Table S9), but the potential impact of background selection is difficult to quantify without knowledge of sex-specific recombination rates and the selection and dominance coefficients of loci under selection. Ancestral selective sweeps on the X could also have contributed to the reduced X differentiation if the same variants were selected or spread across populations (Nolte et al., 2013). Different selective sweeps on the X within both

species could contribute to the greater species differentiation at X-loci. However, recent or frequent bouts of sweeps on the X would be expected to lower the relative X/A diversity (<0.75), which was not the case (Garrigan et al., 2014).

5 | CONCLUSIONS

Sex chromosomes are predicted to exhibit reduced diversity and greater divergence between species and populations compared to autosomes, due to differences in the efficacy of selection and drift on these regions (reviewed in Schaffner et al., 2004, Vicoso & Charlesworth, 2006, Ellegren et al., 2009; Mank et al., 2010). Here we compared genetic variation at X and autosomal loci within and between two Australian *Teleogryllus* field cricket species and quantified the relative diversity and divergence of putative X and autosomal markers. Our results are unusual in two respects. Although X-loci in interspecific comparisons exhibited increased differentiation compared to autosomes, in line with previous theoretical and empirical evidence, in intraspecific comparisons population differentiation was substantially reduced at X loci compared to autosomes, contrary to expectation. Moreover, intraspecific analyses revealed higher than expected (under neutral assumptions) X-to-autosomal diversity (X/A). These features are consistent with a prominent role for X loci in the development of barriers to reproduction.

Most studies that detect deviations from neutral expectations of X/A divergence tend to find reduced X diversity accompanied by increased population differentiation compared to autosomes, in contrast to our findings. Sex-biased processes, such as strong sexual selection and a high variance in male reproductive success are likely to have played an important part in the higher than expected level of X/A diversity in *Teleogryllus* spp. Although populations of both species have

undergone recent population expansions roughly straddling the Pleistocene-Holocene boundary (17,000 – 5,000 B.P. depending on mutation rate), the strength of these size changes were not sufficient to account for the inflated X/A diversity. Further work should examine whether other demographic and selective process, which can alter the relative diversity of autosomes and X chromosomes, such as sex-biased dispersal or background selection also contribute to the unexpected level of X/A diversity in *Teleogryllus* spp. Research into the causes and consequences for the inflated X/A diversity in these species might provide important insights into the processes involved in the evolution of X chromosomes, and their contributions to the establishment and maintenance of reproductive isolation.

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DATA ACCESSIBILITY

All demultiplexed read data used for genotyping are archived on the European Nucleotide Archive: BioProject ID PRJEB26502. Python scripts and VCF files containing all variants and filtered autosomal and X-linked SNPs are available on dryad. Dryad doi:10.5061/dryad.573531d

AUTHOR CONTRIBUTIONS

P.A.M, S.P and N.W.B. designed the study. P.A.M and N.W.B. performed fieldwork. S.P. prepared DNA for RAD-sequencing at Edinburgh Genomics. T.C. and J.E.R. performed initial bioinformatic analyses to construct *de novo* assemblies and call and filter SNPs, and established the initial method for filtering X-loci. P.A.M. performed further SNP filtering, population genomic analyses and data visualisation with advice from J.E.R., M.G.R. and N.W.B. The manuscript was written by P.A.M., J.E.R., M.G.R., and N.W.B.

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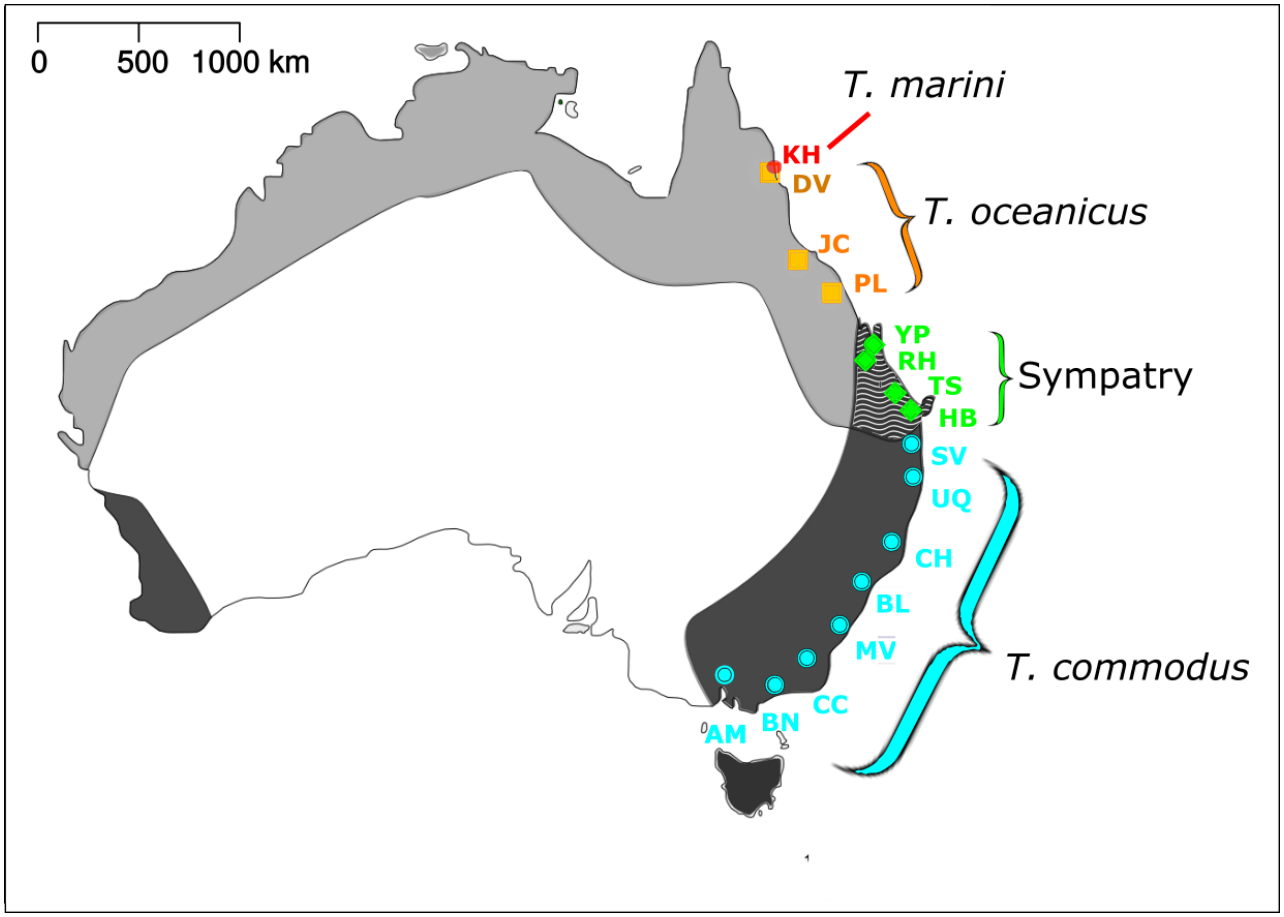

Figure 1 | Species distributions and DNA sampling sites in Australia
T. oceanicus is found across the north (represented by light grey area), while *T. commodus* (dark grey areas) is mainly restricted to the south-eastern coast, but has also been documented in the south west (Otte & Alexander, 1983; Bailey et al., 2017). Both species overlap in an area ~400km long on the mid-eastern coast in Queensland (wavy white lines). Orange squares indicate allopatric populations of *T. oceanicus,* green diamonds represent sympatric populations of both species, and blue circles indicate allopatric populations of *T. commodus.* The red dot in the north indicates the site where we encountered and sampled the putative closely-related species *T. marini* (Otte & Alexander, 1983). Two-letter population codes and sampling details correspond to populations described in Table S1, Supporting Information.

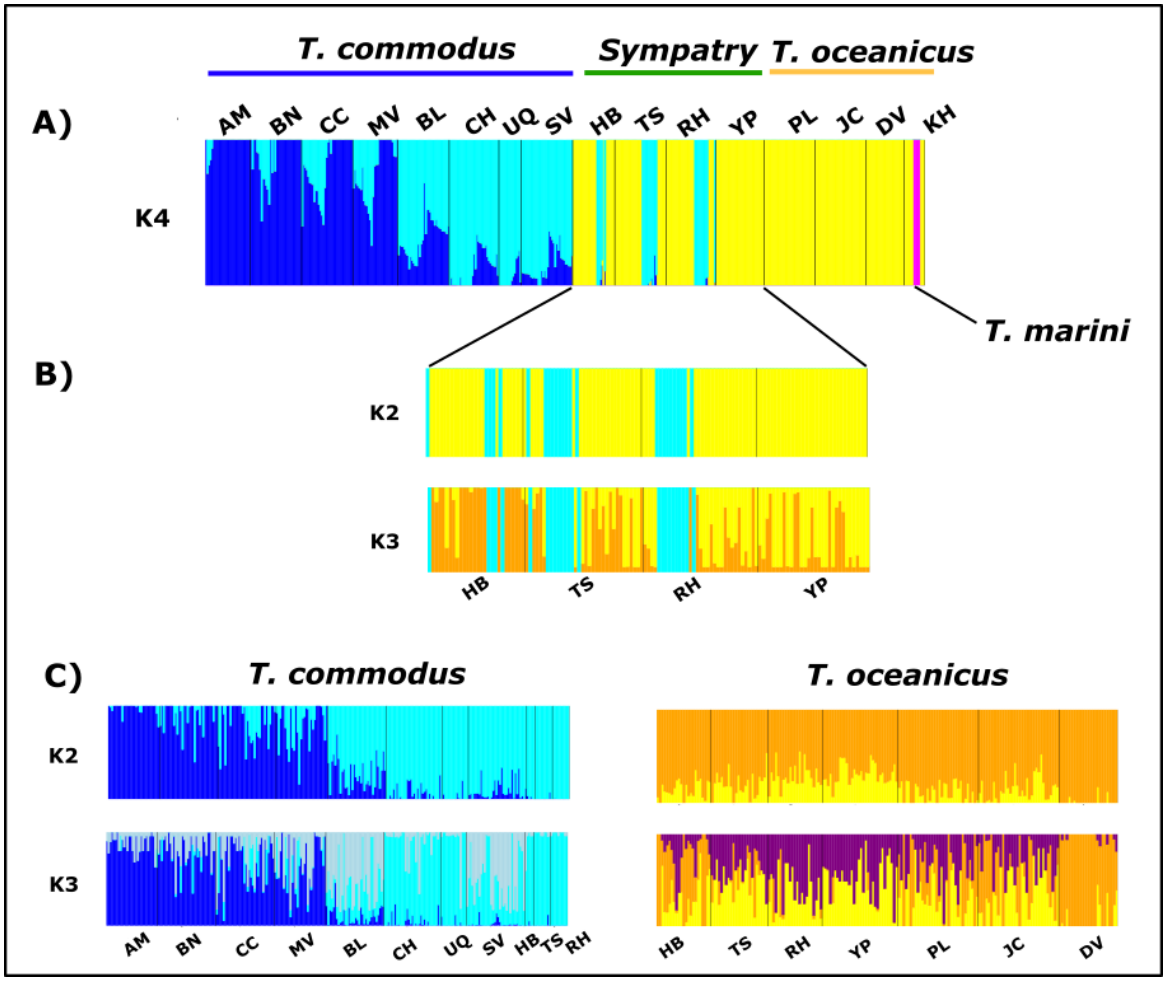

Figure 2 | Population genetic structure within and between *Teleogryllus* species

Plots of individual assignments to the inferred genetic clusters for the most probable K-values for different data subsets. Populations are arranged in latitudinal order from south (left) to north (right). **(A)** Combined species assembly for all populations (n = 478, SNPs = 36,566, K = 4). **(B)** Subset of sympatric populations (n = 127, SNPs = 60,841, K = 2 or 3). **(C)** Fine scaled analysis of intraspecific allopatric and sympatric population genetic structure based on species-specific data subsets in which individuals were assigned to either species group based on the initial analysis (*T. commodus* : n = 269, SNPs = 38,121; *T. oceanicus* : n = 195, SNPs = 41,928, K = 2 or 3).

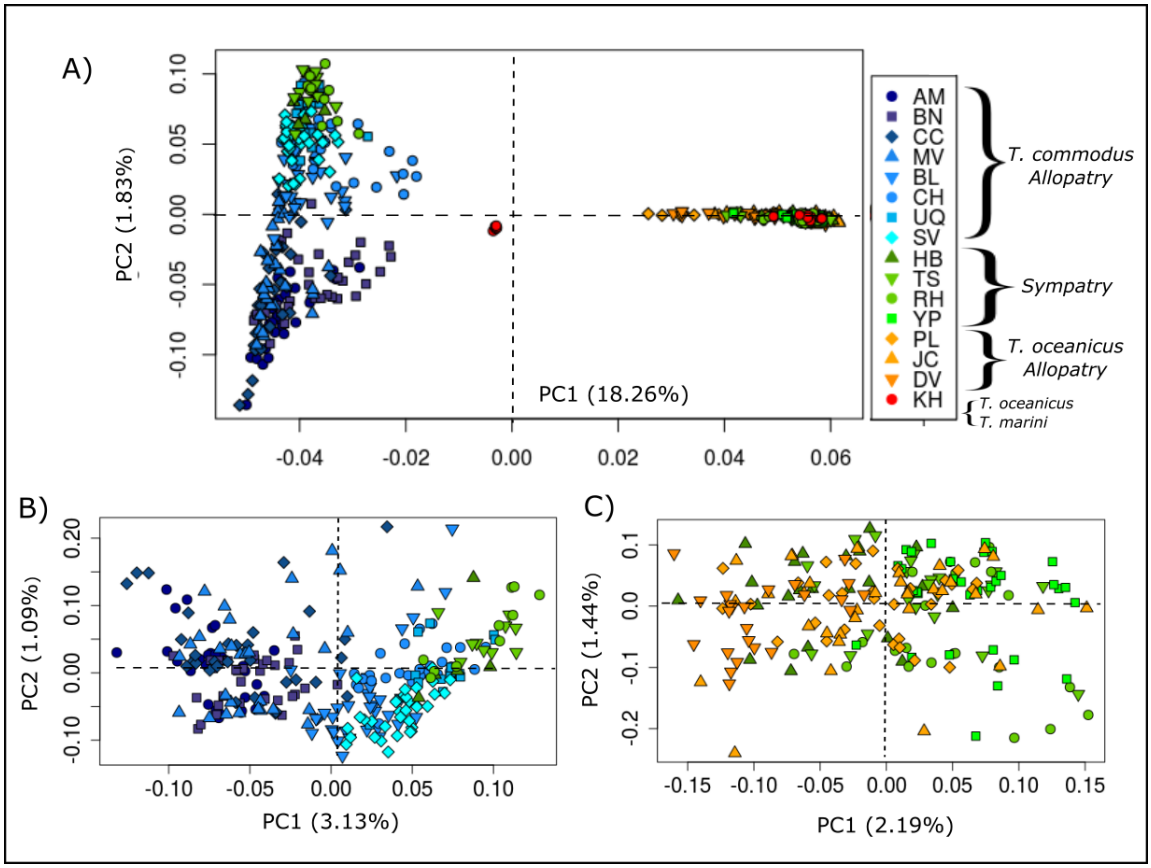

Figure 3 | Genome-wide population genetic structure inferred across populations and species of *Teleogryllus*

PCA for three different data sets. **(A)** Both species encompassing 16 populations (combined assembly: n = 470, SNPs =39,238 (LD-pruned SNPs 23,393)). The LD-pruned SNPs reflects the SNPs retained after removing loci in high linkage disequilibrium. The three clusters correspond to the putative species: *T. commodus*, *T. oceanicus* and *T. marini*. **(B)** *T. commodus* individuals from 11 populations, encompassing 8 allopatric and three sympatric populations (*T. commodus* assembly; n = 265, SNPs = 40,728 (LD-pruned SNPs 26,742). **(C)** *T. oceanicus* individuals from 7 populations (*T. oceanicus* assembly; n = 200, SNPs = 44,941 (LD-pruned SNPs 27,350). Each point represents an individual cricket and colours correspond to sampling sites.

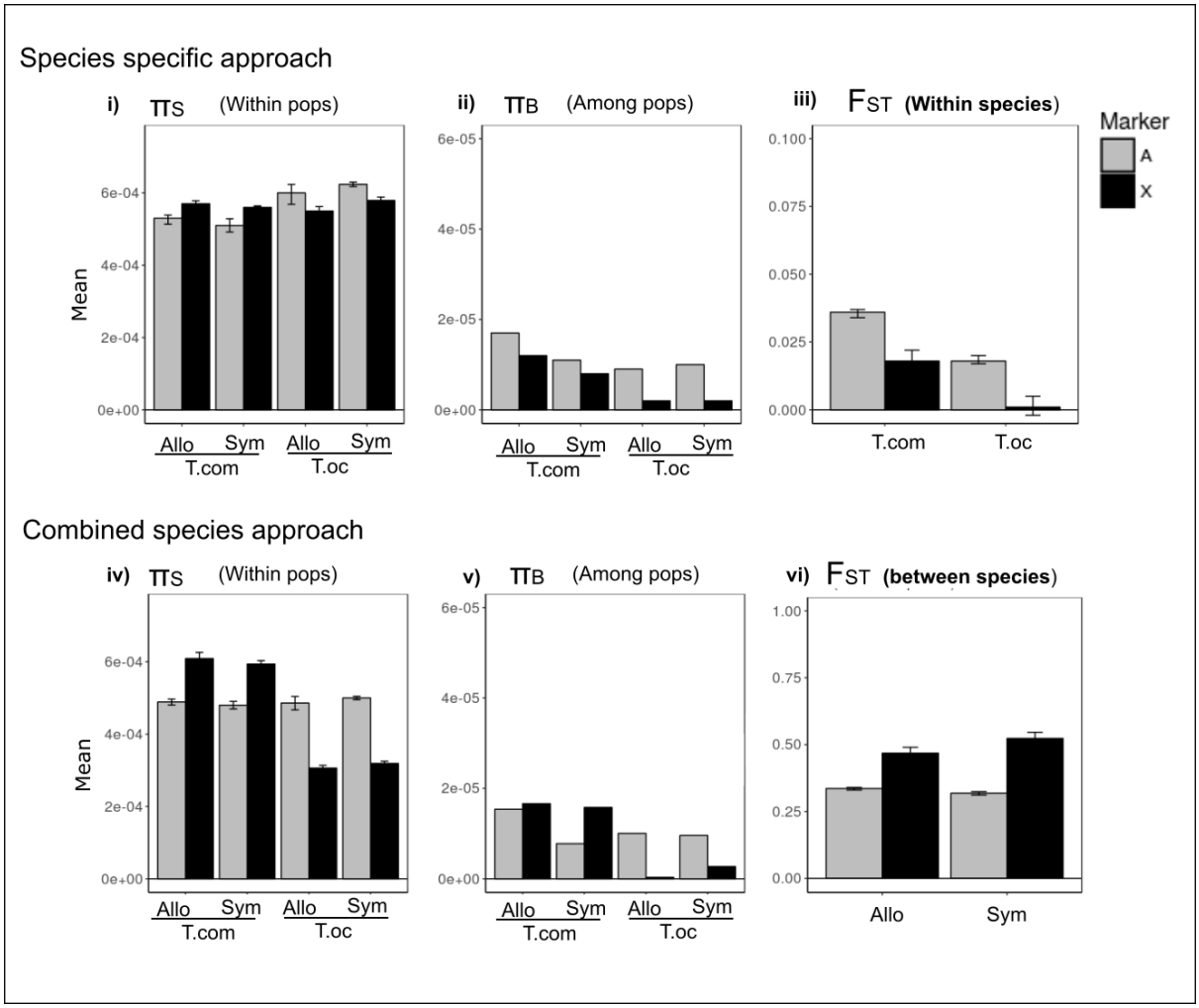

Figure 4 | Genetic diversity and differentiation within and between *Teleogryllus* species at X and autosomal loci

To compare genetic diversity within and between species two SNP datasets were used which differ in the assembly and filtering protocol (detailed in methods section)**. Species specific approach**: i) π_S is the within population diversity ii) π_B is the between population component of diversity within each species. iii) F_{ST} among populations within each species. Intraspecific F_{ST} between allopatric and sympatric regions are omitted for clarity. **Combined species approach**: iv) π_S v) π_T as above with mean values from allopatric and sympatric regions for both species shown. v) F_{ST} between species. Mean values from allopatric and sympatric regions for both species shown. Error bars indicate 95% confidence intervals.

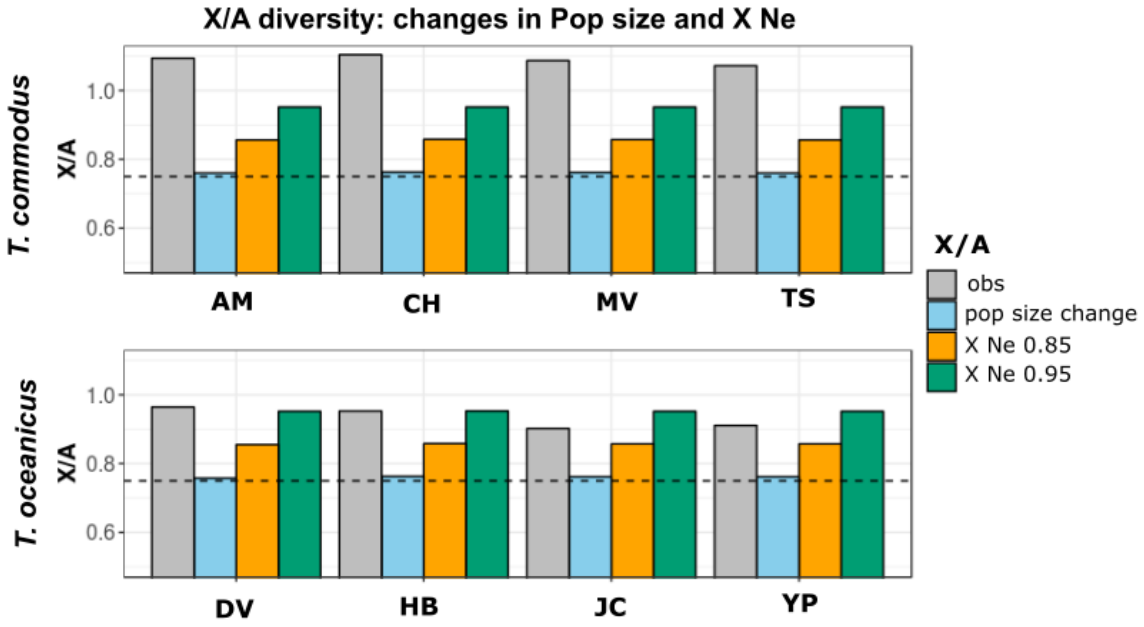

Figure 5 | Observed and expected X/A diversity within *T. commodus* and *T. oceanicus* populations

The observed and expected X/A diversity given changes in population size and Ne for X-loci. The expected X/A diversity was calculated based on demographic parameters estimated using Fastsimcoal (current effective population size and the intensity of a population size change) and equations proposed by pool & Nielsen, (2007) which relate population size changes to X/A diversity (Suppl. Eq. 3). In addition, we adjusted the inheritance factor (h1) for X-loci to encompass other processes which might alter the relative effective population size (Ne) of X and autosomal genes. We calculated expected X/A diversity using three different values of h1, representing differences in the female effective population size. Under neutral assumptions of an equal sex-ratio of breeding individuals h = 0.75, whereas higher h values (e.g. h = 0.85 - 0.95) represent a large female-biased Ne. The dashed line indicates the expected neutral level of X/A diversity 0.75. Comparisons are based on a subsample of populations, encompassing the broad geographical range of both species.

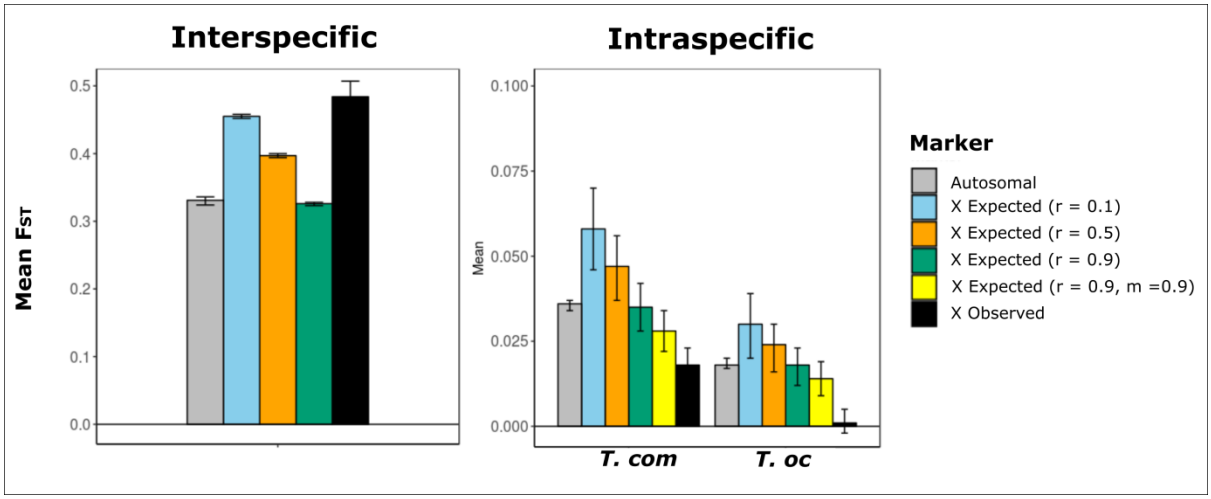

Figure 6 | Observed and expected differentiation at X-loci within and between *T. commodus* and *T. oceanicus*

The expected mean F_{ST} for X-markers was calculated based on autosomal F_{ST} given a particular effective population size (Ne) and female-biased migration rate, using formulas proposed by Ramachandran et al., (2004) and Ségurel et al., 2008 (Suppl. Eq. 1, Eq. 2, Supporting Information). We calculated expected F_{ST} for X loci using three different values of r (the female fraction of the effective population size), encompassing the full range of sex ratios: r = 0.5 for an equal mix of males and females, r = 0.9 for an extreme female bias, and r = 0.1 for an extreme male bias. In addition, we examined the effect of a strong female-biased migration rate (mf=0.9). Interspecific F_{ST} values (left panel) were based on comparisons between eleven *T. commodus* populations (three of which are sympatric) and eight *T. oceanicus* populations (four are sympatric), whereas intraspecific F_{ST} values (right panel) were based on comparisons among the respective populations within each species. Error bars indicate 95% confidence intervals.

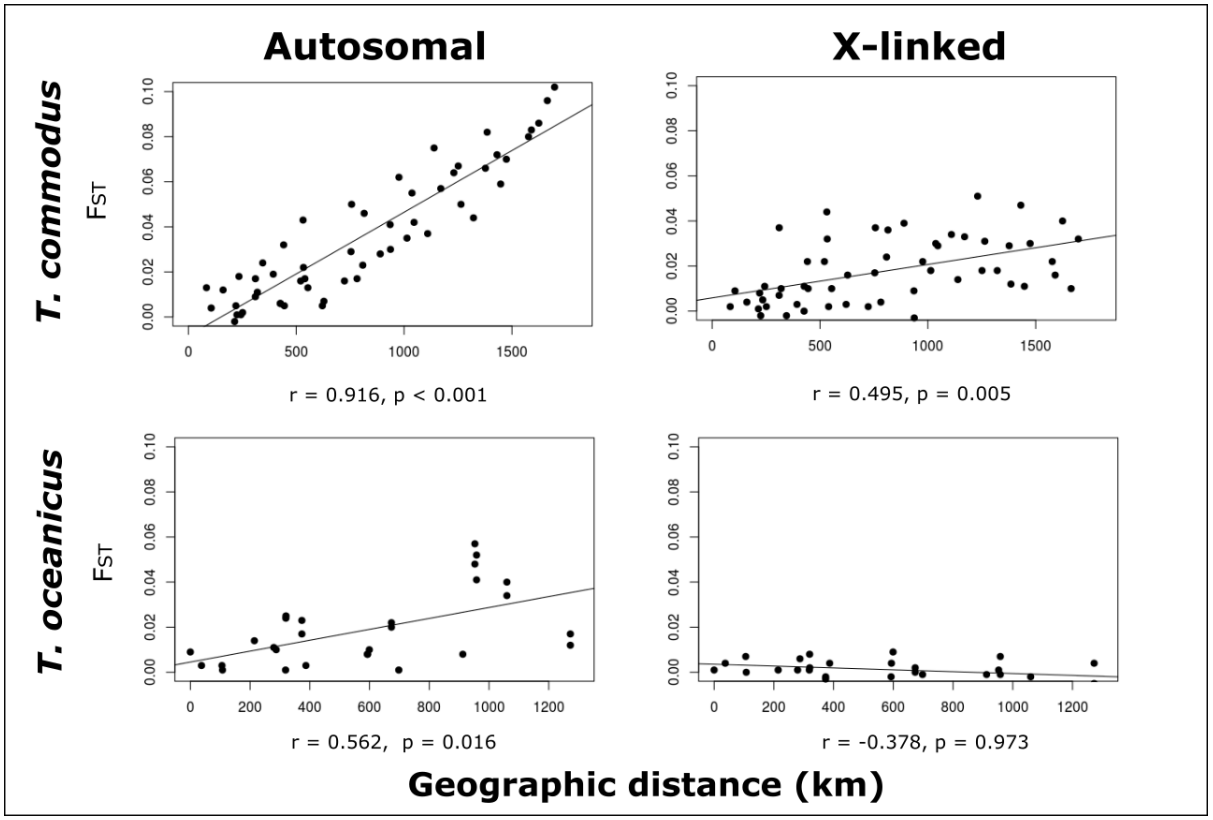

Figure 7 | Isolation by distance in both species for X and autosomal markers Relationship between population genetic differentiation (mean F_{ST}) and geographic distance (Euclidean) at autosomal and X-linked markers (left and right columns, respectively) for *T. commodus* and *T. oceanicus* (top and bottom rows, respectively). Comparisons between the species for the slopes and intercepts are in the supplementary (Table S10).

Table 1 | Population genetic summary statistics for autosomal and X-loci

Ho is the observed heterozygosity, total nucleotide diversity (π_T) was partitioned into within-population diversity (π_S) and the between-population component (π_B). F_{IS} is the inbreeding coefficient, which can range from -1 to 1 (low inbreeding - high inbreeding, respectively). The ratio of X to autosomal diversity (X/A) is provided and the 95% confidence intervals (CI) are in parentheses. Mean F_{ST} is shown, with 95% CI based on 1,000 bootstraps in parentheses. "Area" indicates allopatry ("allo") or sympatry ("sym"). Based on the A1 and X1 filters and only females were included to avoid bias introduced by male hemizygosity.

			(♀)	Autosomal loci			X-linked loci		
	Pop	**Area**	**n**	***Ho***	π_S	F_{IS}	***Ho***	π_S	F_{IS}
T. commodus	**AM**	*Allo*	16	0.213	0.529×10^{-3}	0.063	0.223	0.578×10^{-3}	0.076
	BN	*Allo*	17	0.223	0.526×10^{-3}	0.027	0.233	0.569×10^{-3}	0.032
	CC	*Allo*	17	0.215	0.552×10^{-3}	0.094	0.224	0.581×10^{-3}	0.085
	MV	*Allo*	16	0.215	0.547×10^{-3}	0.083	0.233	0.595×10^{-3}	0.067
	BL	*Allo*	17	0.200	0.519×10^{-3}	0.097	0.203	0.548×10^{-3}	0.102
	CH	*Allo*	13	0.205	0.494×10^{-3}	0.044	0.217	0.545×10^{-3}	0.058
	UQ	*Allo*	10	0.206	0.515×10^{-3}	0.062	0.209	0.555×10^{-3}	0.088
	SV	*Allo*	17	0.203	0.526×10^{-3}	0.101	0.215	0.559×10^{-3}	0.083
	HB	*Sym*	3	0.197	0.510×10^{-3}	0.049	0.216	0.562×10^{-3}	0.043
	TS	*Sym*	6	0.197	0.526×10^{-3}	0.107	0.205	0.564×10^{-3}	0.112
	RH	*Sym*	4	0.198	0.494×10^{-3}	0.028	0.217	0.562×10^{-3}	0.054
	Mean			0.207	0.522×10^{-3}	0.069	0.218	0.565×10^{-3}	0.073
	π_T				0.543×10^{-3}			0.578×10^{-3}	
	π_B				0.232×10^{-4}			0.142×10^{-4}	
	X/A (π_S)			1.084 (1.069 – 1.099)					
	F_{ST}			0.036 (0.034 – 0.037)			0.018 (0.013 – 0.022)		
T. oceanicus	**HB**	*Sym*	12	0.218	0.622×10^{-3}	0.105	0.494	0.593×10^{-3}	0.133
	TS	*Sym*	11	0.221	0.617×10^{-3}	0.082	0.515	0.578×10^{-3}	0.085
	RH	*Sym*	12	0.219	0.624×10^{-3}	0.103	0.526	0.571×10^{-3}	0.084
	YP	*Sym*	17	0.219	0.632×10^{-3}	0.122	0.510	0.576×10^{-3}	0.104
	PL	*Allo*	15	0.227	0.607×10^{-3}	0.054	0.526	0.527×10^{-3}	0.067
	JC	*Allo*	17	0.215	0.622×10^{-3}	0.121	0.494	0.561×10^{-3}	0.107
	DV	*Allo*	15	0.220	0.556×10^{-3}	0.011	0.212	0.537×10^{-3}	0.033
	KH	*Allo*	8	0.216	0.599×10^{-3}	0.075	0.171	0.559×10^{-3}	0.203
	Mean			0.219	0.610×10^{-3}	0.084	0.202	0.563×10^{-3}	0.102
	π_T				0.622×10^{-3}			0.566×10^{-3}	
	π_B				0.126×10^{-4}			0.243×10^{-5}	
	X/A (π_S)			0.923 (0.902 – 0.944)					
	F_{ST}			0.018 (0.017 – 0.020)			0.001 (-0.002 – 0.005)		

Table 2 | The strength and significance of correspondence between autosomal and X-loci and tests for isolation by distance

Species	Marker	n	SNPs	A vs. X	Geographic association
T. com	Autosomal	265	26447	r = 0.535, p = 0.004**	r = 0.916, p < 0.001***
	X-linked	265	2405		r = 0.495, p = 0.005**
T. oc	Autosomal	200	34010	r = -0.227, p = 0.821	r = 0.562, p = 0.016 *
	X-linked	200	1288		r = -0.378, p = 0.973

Significance tested using 10,000 permutations (* p < 0.05, ** p < 0.01, *** p < 0.001).

Table 3. | **Demographic parameter point estimates** and 95% confidence intervals in parentheses calculated in Fastsimcoal2

Pop	SNPs	NPOP	NANC	TEXP	NPOP/NANC
AM	16992	363213	36445	34776	9.97
		360565.3 - 364606.1	36228.7 - 36643.7	34551.7 - 34897.4	
MV	21666	351480	39250	35139	8.95
		347863.2 - 351955.9	38949.5 - 39364.9	35029.8 - 35383.6	
CH	16370	350943	27536	40774	12.74
		348602.3 - 352647.5	27181.1 - 27590.0	40696.3 - 41108.8	
TS	18731	373993	38598	34627	9.69
		369303.8 - 373741.1	38394.1 - 38846.7	34435.5 - 34824.3	
HB	19007	294634	43362	39854	6.79
		292443.6 - 294913.5	43116.8 - 43621.3	39610.8 - 40141.9	
YP	19712	308630	48041	37717	6.42
		305470.3 - 309170.1	47493.3 - 48021.3	37692.8 - 38239.4	
JC	18865	289760	42935	42274	6.75
		286936.2 - 289770.7	42583.4 - 43116.6	42074.4 - 42663.5	
DV	14793	482748	34621	35108	13.94
		475546.4 - 481149.8	34367.6 - 34743.3	35042.54 - 35351.1	

Demographic parameter estimates are from the model with the highest likelihood which in our case was a model of population expansion. NPOP is the current effective population size, NANC is the ancestral population size and TEXP is time in generations ago when exponential growth started. 95% confidence intervals were estimated from 100 parametric bootstraps (20 runs and 100,000 simulations per simulated site frequency dataset).